

## The Use of Infrared Spectroscopy in the Investigation of Urolithiasis

### Utilizarea spectroscopiei în infraroșu pentru investigarea urolitiazii

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#### Abstract

*Infrared (IR) spectroscopy is a modern physical-chemical method suitable for the investigation of kidney stones composition. The application of this method in our work was anticipated by performing the standard infrared-spectra on pure compounds: oxalic acid, oxalates, phosphate, carbonate, uric acid, and xanthine. This work reveals aspects regarding urolithiasis types by Fourier Transform Infrared (FTIR) Spectroscopy method. The purpose of this paper is to contribute to the determination of the urolithiasis composition correctly correlated with specific pathophysiological conditions. These results permit further investigation on the uroconcrements for which we have established the urolithiasis type and enable us to determine their metal concentration by atomic absorption spectroscopy. The data regarding the urolithiasis composition are important for clinical guidelines in the prophylaxis and methaphylaxis of urolithiasis.*

**Keywords:** urinary calculi, FT-IR spectroscopy, qualitative composition, etiology

#### Rezumat

*Spectroscopia în infraroșu (IR) este o metodă fizico-chimică adecvată pentru investigarea compoziției calculilor renali. În investigațiile noastre, aplicarea acestei metode s-a realizat utilizând înregistrările spectrelor compușilor puri de: acid oxalic, oxalați, fosfați, carbonați acid uric și xantină. Această lucrare prezintă aspecte referitoare la tipurile de urolitiază folosind metoda spectroscopie în infraroșu cu transformata Fourier (FT-IR). Scopul acestei lucrări este de a contribui la determinarea compoziției urolitiazii corelată corect cu condițiile patofiziologice specifice tipului de urolitiază. Aceste rezultate permit investigații ulterioare ale uroconcrementelor pentru care s-a stabilit tipul acestora, având ulterior posibilitatea de a determina concentrația metalelor din acești calculi prin spectrometria de absorbție atomică. Datele cu privire la compoziția urolitiazelor sunt importante în management-ul clinic, în profilaxia și metafilaxia urolitiazelor.*

**Cuvinte cheie:** calculi urinari, spectroscopie FT-IR, compoziție calitativă, etiologie

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## Introduction

The metabolic and urodynamic disturbances of various organic and inorganic compounds in the urinary medium induce urolithiasis. Among the factors that can lead to urinary stone formation and can influence its characteristics we can mention sex and age, etiological factors such as metabolic disorder, ionic transport within the intestinal tract and in the kidney, diet including fluid intake, geographical location and climate etc. Urolithiasis is a common urologic disease whose prevalence is about 1-20% and increasing throughout the world. About 2-5% of the population in Asia, 8 – 15% in Europe and North America and 20% in Saudi Arabia develop kidney stones in their lifetime (1- 5). Renal stone tends to recur, and the rate of recurrence is about 75% during 20 years (6).

Nephrolithiasis (urinary calculi) represents a concretion composed of crystalline components and organic matrix (7). Though the symptomatic presentations may be similar, the disorder is heterogeneous regarding composition and etiology.

Chemical methods are rather unsatisfactory for the analysis of urinary calculi, because they are time-consuming; they necessitate large stone samples and cannot distinguish between the two commonly occurring calcium stones (monohydrate/dihydrate). Also, these methods do not yield information about the crystalline phases and do not detect rare drug-induced or metabolic compounds, such as 2,8-dihydroxyadenine (8), xanthine or silica. Physical-chemical and mineralogical tools are among the methods that are increasingly used in order to establish the composition of urinary calculi.

Infrared spectroscopy investigation of surgically extracted and spontaneously eliminated calculi allows us to find out their qualitative composition. The interpretation of spectra by means of implementing a computer program facilitates defining the types of urolithiasis.

The compounds present in urinary calculi may be of organic nature (oxalic acid, uric acid, xanthine, cystine, cholesterol) or/ and inorganic nature (phosphates, carbonates). The determination

of the pathological conditions involved in stone formation must be based on a careful examination of the structure, and also on the identification of the qualitative and quantitative crystalline composition of urinary stones. Relevant data regarding the structure and the crystalline composition can be found by using two physical methods. For the morphological study we have used a stereomicroscope and, for the molecular and crystalline identification, the Fourier Transform Infrared Spectroscopy (FTIR) method. FTIR, a powerful analytical tool, is able to find all the crystalline, amorphous or poorly crystallized components, and also the mineral and organic molecules. Moreover, it can make distinction between the different crystalline phases of a substance and it can quantify the relative constituent proportions within a heterogeneous stone (9).

Frequently, stone growth is a discontinuous process whose cause cannot always be identified. A transient, intermittent or permanent disorder of urine composition induces urine super-saturation and, as a result, causes urinary calculi. Calculus formation occurs gradually, nucleation being the first step, followed by the growth around the nucleus. Stone growth may be caused by different factors than those causing nucleation (10). It is also possible that the physicochemical background causing stone nucleation does not exist anymore at the time of the patient's investigation. However, the calculus core, cross-section and surface can be separately examined and can provide information about the litho-genetic conditions involved both in the nucleation process and in the stone growth. Such details are essential when reconstructing the history of the calculus formation. Stone composition and structure can provide information on the past conditions that have led to the crystallization process and, thus, on the etiology.

## Materials and Methods

Our study has been performed on 52 calculi using FTIR spectroscopy, which is a physical-chemical method. We have determined the qualitative composition of the uroconcrements obtained from the renal stone patients, hospitalized in the Clinic of

Urology of the University of Medicine and Pharmacy in Timișoara. For the macroscopic study we have used a stereomicroscope. For the morphological study we have used a magnifying glass and a stereomicroscope. The nature of urinary calculi cannot be accurately identified only by a preliminary examination; that would not be enough because it may sometimes lead to confusion (11, 12). It is possible that calculi with different compositions have a similar appearance. Cations and anions in the urinary calculi have been identified by qualitative wet chemical methods (routine techniques) commonly used in Biochemistry laboratories (11). These methods, also called orientative methods, consist in the use of calculus powder. For this purpose, concentrated inorganic acids ( $\text{HNO}_3$ ,  $\text{HCl}$ ) are employed, highlighting the calculus constituents (urate, oxalate, carbonate or phosphate, the ammonium group, Ca and Mg, cystine, xantine). In addition, quantitative determinations of different calculus elements or groups can be made, especially Ca, Mg, phosphate and oxalate. For the determination of calcium, magnesium, and inorganic phosphate the standard procedure can be employed. For instance, calcium can be determined by automatic colorimetry using cresolphthalein complexone as indicator and 8-hydroxyquinoline to suppress magnesium interference. Another way of determining calcium may be by atomic absorption spectrometry, using lanthan chloride to overcome the suppression of calcium absorption by phosphate ion. The most convenient method for the determination of magnesium and other metallic ions is atomic absorption spectrophotometry. With regards to inorganic phosphate, it can be determined by either using the manual phosphomolybdate method or the automatic colorimetry, and UV-VIS spectrometry. A simple permanganate titration can be employed to determine the oxalate, provided that certain precautions are taken. Beside this method, UV-VIS spectrometry can also be used in this case.

FTIR spectra of all urinary calculi were recorded as potassium bromide (KBr) pellets using a Jasco FT-IR 610 Spectrometer (Japan) in the Institute for Research in Chemistry "Raluca Ripan", within the "Babeș - Bolyai"

University. This method requires a small sample of 0.5-2 mg.

Within the first stage, the calculi were repeatedly washed with distilled water, and then dried. Afterwards, each calculus was cut with the help of a fine saw in two equal parts; these parts were examined with a magnifying glass and looked at under a microscope. When the examined section showed a nucleus or other distinctive areas, its parts were studied separately. The samples were potassium bromide (IR purity) compressed. The spectral range was between 4000 and  $400\text{ cm}^{-1}$ .

Due to stretching or bending vibrations of the characteristic groups from the molecules, IR spectra contain absorption bands at different wavelengths. Molecular composition and crystal structure influence the number, the position or the intensity of the absorption bands from the spectrum.

In a preliminary stage of our research, in order to find out the type of urolithiasis, we proceeded to the recording of standard IR spectra of the chemically pure compounds found in calculi: organic (oxalic acid, uric acid, xanthine, cholesterol) and inorganic (phosphates: carbonate apatite, magnesium ammonium phosphate hexahydrate, calcium hydrogen phosphate dihydrate, tricalcium phosphate, and carbonates: calcite- calcium carbonates). We used Merck and Alfa-Aesar compounds. Carbonate apatite (CAP) is a calculus which was chosen out of a multitude of calculi analyzed by FTIR, by comparing the obtained IR spectra with the one belonging to CAP registered in literature (in the IR atlases) (13, 14).

## Results and Discussions

The spectrograms of the chemically pure substances were considered "standard" spectra. By comparing and superposing the calculi spectra with "standard" spectra, we have determined the qualitative and semi-qualitative composition of urinary stones. The intensity of IR bands displays (semi-quantitative) information regarding the degree to which a component's content is found in the calculus (if the compound concen-

tration in the calculus is low, the result will be a specific vibration of low intensity).

The IR absorption of the studied sample has shown the presence of the oxalate, uric acid and/or hydroxyapatite groups in the urinary calculi. The IR spectrum band of 1620  $\text{cm}^{-1}$  (15) and that of 1316  $\text{cm}^{-1}$  (15), 1315  $\text{cm}^{-1}$  (16) of the pure synthetic monohydrate calcium oxalate correspond to the symmetric and asymmetric C–O stretching of coordinated oxalate groups. In our case, the corresponding values for calcium oxalate are 1622  $\text{cm}^{-1}$  and 1319  $\text{cm}^{-1}$ . For calculi, the stretching frequencies characteristic to the oxalate have been found at 1621  $\text{cm}^{-1}$  and 1320  $\text{cm}^{-1}$  ( $\nu\text{C-O}$ ).

The degree of hydration of different substances can be given by the presence or absence of some bands from the IR spectrum; therefore, for COM (calcium oxalate monohydrate), this degree is indicated by the two peaks between 850 and 950  $\text{cm}^{-1}$  (for COD these two peaks do not appear). In the 3500–3000  $\text{cm}^{-1}$  region a broad spectrum appears for COD (calcium oxalate dihydrate), by contrast with the one for COM, which contains four weak peaks (*see Figure 2, traces B, C*). For the mixture calculi types, these differences cannot be very clearly observed and can be mistaken because of the overlapping of the bands characteristic to different constituents, and for this reason the second derivative has been used. The strong peaks around 1620  $\text{cm}^{-1}$  and the weak band at 661  $\text{cm}^{-1}$  can be assigned to the bending and wagging modes of the water molecules (16, 9). The bands at 1622 for (COM)/or 1645 (for COD), 1319, 780 and 517  $\text{cm}^{-1}$  are due to  $\nu\text{a}(\text{C=O})$ ,  $\nu\text{s}(\text{C=O})$ ,  $\delta(\text{O-C=O})$  and  $\nu(\text{Ca-O})$ , frequencies of the oxalate part (17).

IR spectrum of the uric acid is characterized by many bands indicated by the purinic ring at 3138, 3020 and 2836  $\text{cm}^{-1}$  (absorption bands). The bands are due to N–H stretching vibrations, and also, there are additional bands at 1351, 1124, 1029 and 786  $\text{cm}^{-1}$  wave numbers. In the case of urinary calculi, the N–H stretch-

ing vibrations appear at 3142, 3026, 2857  $\text{cm}^{-1}$  values and there are also IR absorptions at 1353, 1126, 1031 and 788  $\text{cm}^{-1}$ , confirming the presence of uric acid in the calculi.

Stretching frequencies of 567, 603, 985, 1038 with a shoulder at 1105  $\text{cm}^{-1}$  correspond to the apatite mineralogical form of the calcium phosphate. The phosphate ion from CAP/ HAP yields a strong and broad absorption between 1100 and 1000  $\text{cm}^{-1}$ . We can attribute  $\nu_3(\text{PO}_4)$ , lattice mode and  $\nu_4(\text{PO}_4)$  to the 3 major peaks of the phosphate anion at 1038, 603 and 567  $\text{cm}^{-1}$  frequencies. For the determination of the calcium carbonate we have taken into account absorption values specific to it, at 1415 and 875  $\text{cm}^{-1}$ . Carbonate apatite is characterized by the 1422 and 1652  $\text{cm}^{-1}$  IR bands, approximately. Also, X-ray diffraction study confirmed the presence of HAP/CAP. Struvite has a characteristic infrared spectrum and is easily recognized even in mixed stones both by the position of the strong absorption band at 1008  $\text{cm}^{-1}$  (which is due to the absorption of phosphate group) and by the presence of other bands at 2377, 1476, 1437, 872, 770 and 574  $\text{cm}^{-1}$  (18). The bands at 1476 and 1437  $\text{cm}^{-1}$  can be attributing to vibration of  $\text{NH}_4^+$  group.

When struvite is in association with apatite, the presence of the latter can be deduced by absorption at 600  $\text{cm}^{-1}$  and by a shift of the band at around 1010  $\text{cm}^{-1}$  towards higher frequencies.

The results obtained by FTIR spectroscopy on 52 uroconcrements are presented in *Figure 1*. From all the urolithiasis cases analysed by us, there were 11 cases of simple urolithiasis and 41 of mixed urolithiasis (binary, ternary, quaternary). Among the cases of simple urolithiasis, 8 were composed of calcium oxalate and 3 of uric acid anhydrous (UA). Calculi from 33 mixed urolithiasis had oxalate (63.46%) and uric acid was found in 11 mixed calculi (21.15%). Phosphate was found in numerous calculi (27 cases - 51.92 %), especially in mixture with oxalate (19 calculi - 17.30 %), but the concentration of phosphate was more or

less than the oxalate one (see *Figure 1*). One case of calculus was a mixture between oxalate, phosphate and uric acid. In the category of mixed urolithiasis we have also included the urolithiasis with one component found in different hydration phases (crystallization lattices).

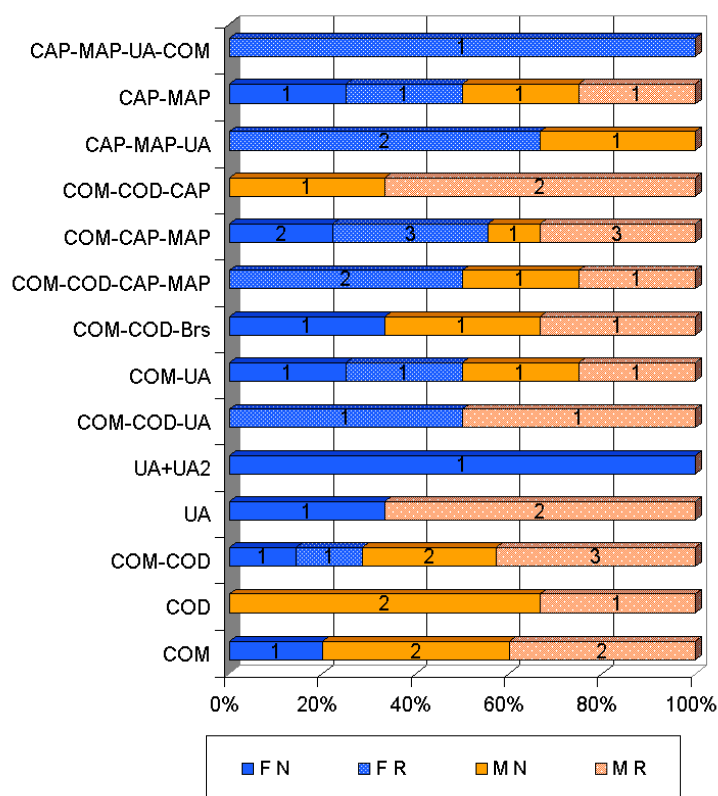
We can conclude that 40.38% of the cases (21 calculi of 52) belong to female patients, while the rest of 59.62% belong to male patients. 61.54% of the cases represent recidivating urolithiasis. The proportion between the number of urolithiasis cases at first apparition and the recidivating urolithiasis cases (N:R) is approximately equal in men and women (0.72 - men, 0.75 - women).

There are different forms in which the same chemical component may crystallize: for

example, calcium oxalate may be present in the form of calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) or very rarely calcium oxalate trihydrate (COT). Often, these appear with their mineralogical denominations: whewellite for calcium oxalate monohydrate (Wh); whedellite for calcium oxalate dihydrate (Wd); humboldtine for iron oxalate dihydrate. Uric acid is found in two types of crystallisation: anhydrate (UA) and uric acid dihydrate (UA2). Calcium phosphate is found in multiple forms: calcium phosphate, calcium hydrogen phosphate dihydrate (brushite), calcium hydrogen phosphate, apatite like carbonate apatite (CAP) and hydroxyapatite (HAP), octacalcium phosphate a.o. From all the forms in which calcium phosphate can be found, the one which mostly occurs is carbapatite (a carbonate calcium phosphate crystallized in the hexagonal system). In urinary calculi phosphate can be found not only as calcium phosphate but also as magnesium ammonium phosphate hexahydrate (MAP). Mineralogical denominations of CAP and MAP are dahllite and struvite, respectively.

Different biochemical conditions can cause several crystallization forms (19). Consequently, accurate stone analysis must determine not only the molecular species of the calculus, but also the crystalline forms of the chemical constituents. The majority of stones are heterogeneous calculi, i.e. their composition is mixed. From them, approximately 80% contain a mixture of CaOx and/or phosphate at different levels (in our case 78.9%).

*Figures 2, 3 and 4* compare the IR spectra of various stones with different compounds commonly found in the constitution of a renal



**Figure 1. Distribution of calculi based on composition, urolithiasis type and sex of the patients from whom the calculi have been extracted (M- male; F- female; N- first time urolithiasis; R - recidive urolithiasis)**

calculus (called standards). Similarities can be observed between the A, B, C, and D spectra, and between F and G spectra. Also, B spectrum is similar to E and F spectra, and J spectrum resembles K and L spectra.

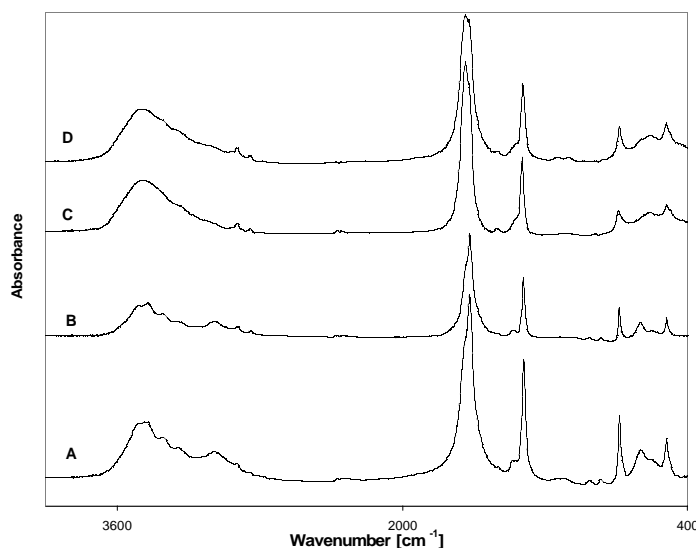
Moreover, there is an overlap between H and J spectra. Note that in these figures B, C, F, J and K represent the spectra of the pure compounds (of the standards): B - COM spectrum, C - COD

spectrum, F - spectrum of uric acid monohydrate, J - CAP spectrum and K - MAP spectrum.

From *Figure 2* it can be concluded that a calculus with an A spectrum contains calcium oxalate monohydrate (the A and B spectra are very well superposed). In the case of a calculus with a D spectrum, it is questionable whether or not this stone is a mixture of COM and COD. The D spectrum looks like C, but between D and B spectra there are some superposing peaks. It becomes certain that this stone is a mixture of COM and COD when superimposing the second derivative of the spectra (the calculi spectra with the standard ones: COM and COD).

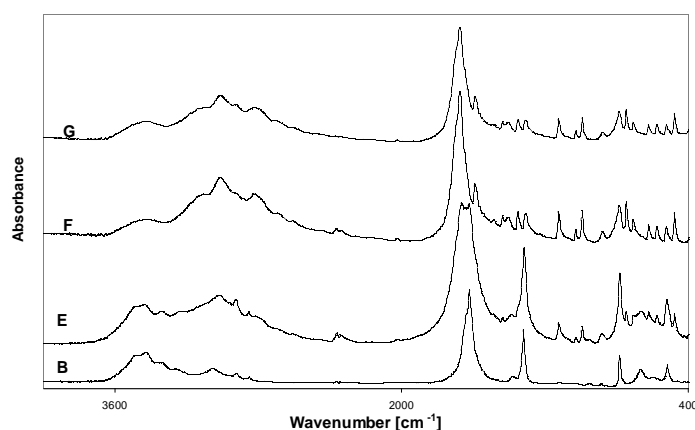
From *Figure 3* it can be concluded that in the composition of an E stone there are both uric acid and calcium oxalate monohydrate. By superimposing the second derivative of the spectra (the calculi spectra with the standard ones: COM and UA), we know that this stone is a mixture of COM and UA (b spectrum). We have also used the X-ray diffraction method for some calculi in order to check the derivation method (of the second derivative). The second derivative helps us identify the nature of the hydration for the calculus components. This is because it shows notable differences on specific intervals, for mixtures of compounds found in various hydration states, compared to the IR spectra, which look somewhat similar.

The D spectrum (*Figure 2*) of the stone sample was subjected to the calculation of the second derivative of absorbance curve in order to determine whether it contains only COM or it is a mixture of COM and COD. This is because the period 3600-2900  $\text{cm}^{-1}$  is a broad band spectrum different from that of B -



**Figure 2. FT-IR spectra for calculi, for COM and for COD.**

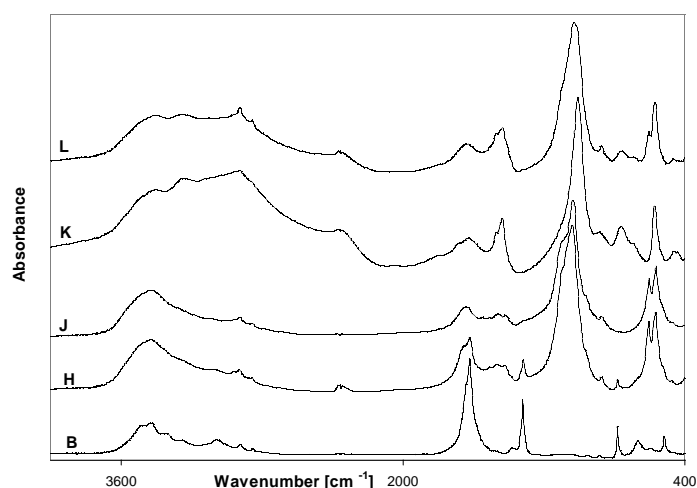
A – simple (COM) calculus spectrum; B – COM spectrum; C – COD spectrum; D – mixed (COM and COD) calculus spectrum



**Figure 3. FT-IR spectra for calculi, for COM and for uric acid.**

B – COM spectrum; E – mixed (COM and UA) calculus spectrum; F – uric acid spectrum; G – simple calculus (with uric acid) spectrum

the COM one (used as a standard). Also, the A sample spectrum was subjected to derivation. In Figure 5, in a particular region of wave number, second derivatives for D spectrum (from Figure 2) are simultaneously compared with the COM standard (Figure 2B) spectrum and the COD standard (Figure 2C) spectrum. Second derivatives are denoted by c (for the calculus) and d for COM. This figure shows similarities between the IR spectra, in contrast with the second derivative where there is a noticeable difference within the 1300-1330  $\text{cm}^{-1}$  region and within the 1200-800  $\text{cm}^{-1}$  region. The appearance of two peaks of similar intensities for the second derivative (Figure 3c) shows that the stone contains a mixture of COM and COD in equal proportions. The peaks centered in 1326  $\text{cm}^{-1}$  and 1317  $\text{cm}^{-1}$  correspond to the COD and COM phase, respectively. The resolution of the second derivative's two peaks in case of the stone with a D spectrum demonstrates this technique's potentiality to determine the components often found in kidney stones. We used second derivation for all samples in order to check the presence of the same compounds in different hydration phases.



**Figure 4. FT-IR spectra for calculi and for CAP, MAP and COM.**

B – COM spectrum; J – CAP spectrum; K – MAP spectrum; H – mixed (CAP+MAP+COM) calculus spectrum; L – mixed (MAP+CAP) calculus spectrum;

Qualitative chemical tests provide the rough indication of the presence of different constituents in mixed stones. Our IR data are qualitatively matched with wet chemical analysis and powder X-ray diffraction. The X-ray diffraction spectra of our samples have been obtained by a DRON-UM1 diffractometer in Bragg-Brentano geometry, having the following characteristics: theta-2 theta assembly, work voltage  $U = 40\text{kV}$ , electric intensity  $I = 30\text{mA}$ , angular range  $2\Theta$ : 100-700, radiation  $\text{CuK}\alpha$ , graphite crystal monochromator on the diffracted beam. This technique requires a great quantity of calculus, by comparison with FTIR spectroscopy.

IR spectroscopy has gained in popularity as a reliable method of in-vitro quantitative stone analysis especially in the last decade. In many stone centers, FTIR spectroscopy is also being used for the prediction of stone fragility prior to shock wave lithotripsy. Although the reliability of newer tests such as CT spectroscopy, NMR spectroscopy, X-ray diffraction, thermogravimetric and chemical/crystallographic stone analysis has been confirmed by several studies, the importance and utility of FTIR spectroscopy as a routine screening test has also been revealed (20).

The aim of this paper is to establish the frequency of different urolithiasis types and calculi composition correctly correlated with specific pathophysiological conditions. Table 1 presents a summarized comparison of the mineralogical composition of stones in relation to physio-pathologic factors. Our purpose is to present a general view on the relationship between pathology, structure and composition in the case of renal calculi. For this reason, the above mentioned table does not only include stones which exclusively contain an oxalate phase, but also a few examples of renal calculi which have not been approached in our paper.

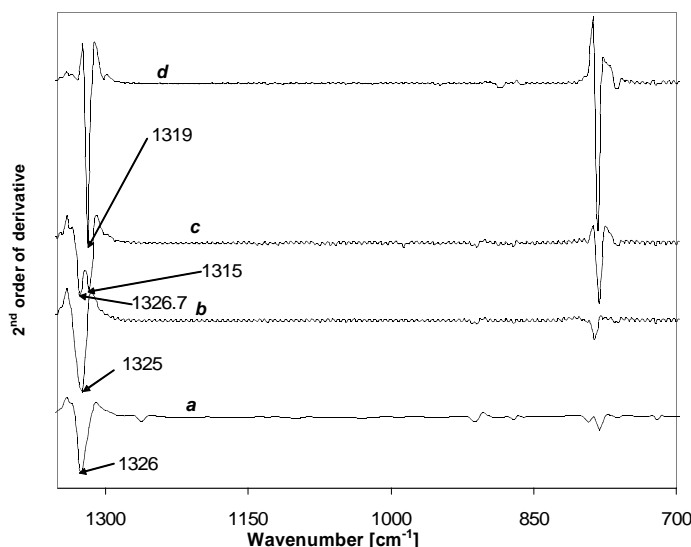
The information collected from the medical records of the pa-

tients from whom the calculi have been extracted reveal that there is a tight connection between the mineralogical calculi composition and the etiologic factors. It has been established that hyperoxaluria is found in 91.1% of the urolithiasis cases with calculi containing COM (calcium oxalate monohydrate). As regards the cases where COM

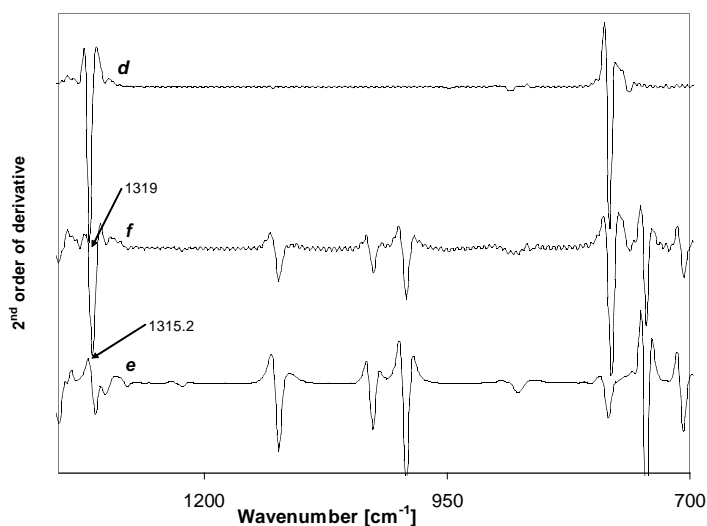
is in multi-component mixture, hyperoxaluria does not exist, which can be explained by the fact that it may have been found in another stage of calculus formation. Urinary infections have been found in 85.4 % of the patients with CAP, MAP or CAP-MAP calculi, due to the fact that the urinary infection has been the cause of calculi formation in

their first stage. In addition, for patients with calculi containing COD it has been discovered that 87.6 % of them had hypercalciuria. All the 4 cases of urolithiasis which developed uric acid calculi have displayed a urinary pH < 5.5. As a result of our analysis, it can be stated that oxalic urolithiasis occurs predominantly in the case of men compared to women, while urinary infection calculi (MAP, CAP) mainly occur in the case of women (Figure 1).

In order to discover the etiology of the urolithiasis, it is important to know the stone chemical composition. Also, information regarding the chemical composition of the urinary calculi helps us prevent the recurrence of the disease and can be used as a base for the therapy in such cases and for a proper disease management. For example, calculi of calcium oxalate dihydrate and struvite are more amenable to disintegration by extracorporeal shock wave lithotripsy than are those composed of cystine (21). The oxalate and calcium concentrations in the urine affect calcium oxalate supersaturation. An important risk factor in stone formation is urinary pH due to several human body processes. A potential mechanism for a permanently low urinary pH in uric acid stone formation can reduce ammonia ( $\text{NH}_3$ ) production, resulting in a decreased buffer capacity for  $\text{H}^+$  ion (caused by the lack of glutamine or by an enzymatic de-



**Figure 5.** The second derivatives of absorbance curve corresponding to: a – COD simple calculus; b – COD standard; c – the second derivative of mixed (COM+COD) calculus; d – COM standard



**Figure 6.** The second derivatives of absorbance curve corresponding to: d – COM standard; e – UA standard; f – mixed calculus (COM+UA)



**Table 1. Correlations between different constituents of calculi and physiopathological factors**

<b>Chemical Composition</b>	<b>Etiological conditions</b>
COM	hyperoxaluria (with or without hyperuricosuria), Cocchi Ricci disease
COD	hypercalciuria (in some cases it can be present hyperoxaluria)
COM+COD	hyperoxaluria + hypercalciuria
UA	intermittent hyperuricosuria + urine at acid pH
UA + UA2	hyperuricosuria + urine at acid pH
AmU	alkalinizing therapy + urinary infection
UA + COM	hyperuricosuria +intermittent hypercalciuria
CAP	urinary infection (non-ureasic) + hyperparathyroidism+ secondary tubular acidosis
MAP+CAP	urinary infection owed to ureasic germs
CAP +MAP+ AmU	urinary infection with urine alkalization
COM +CAP	hypercalciuria + hyperparatiroidism
Protein + MAP + CAP	urinary infections
Protein + COD	Lithiasis due to dialysis
BRS	hypercalciuria + hyperparatiroidism
Cystine	cystinuria

COM-calcium oxalate monohydrate; COD- calcium oxalate dihydrate; UA- uric acid anhydrate; UA2- uric acid dihydrate; AmU-ammonium urate; MAP- magnesium ammonium phosphate hexahydrate (struvite); CAP-(carbonate)apatite; BRS- brushite (calcium hydrogen phosphate dihydrate,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

fect). In our sample struvites are found in a percentage of 51.9% and their formation is due to urinary infection; their treatment involves pharmacological (antibiotic) intervention. The formation of struvite stone is due to urinary infection (of urea splitting microbes) (1, 6, 21). Recurrent infection can be prevented if pH urinary values are kept below 6.0. MAP is produced in alkaline urine almost invariably infected with bacteria containing urease, which converts the urea (found in urine) into ammonia. The pH increase (second to the process mentioned before) within the concentration of the  $\text{NH}_4^+$  ion lead to the formation of apatite.

Furthermore, when the uric acid excretion is very high, the result is the formation of ammonium acid urate. These compounds may accompany struvite in calculi but we have to mention that they happen exclusively in the case of urinary infections.

The pH seems to have a role in the preferential formation of calcium phosphate stones during pregnancy (22).

Experience shows that a detailed analysis of stone structure and compositions is becoming more and more important for identifying the etiology of each stone disease, due to the fact that the growth and the evolution of stones in the urinary tract can sometimes last for several years. When the stone is removed, the causes of stone nucleation and growth cannot be determined and no abnormality is revealed by the chemical studies of blood and urine. That is why a correct analysis of stone structure and composition can enable us to obtain the information above.

From the variety of physical and chemical techniques intended for the kidney stone study, IR –analysis certainly shows the highest degree of accuracy. The improved characteristics of FTIR spectrometers allow an easy analysis of the molecular composition even on very small samples.

The metallograms of urolithiasis can be determined after this preliminary investigation. In other papers, data can be found regarding

metals concentration in various types of simple and mixed urolithiasis (23, 24). The quantum of the main alkaline metals (Na, K), the alkaline-earth metals (Ca, Mg) and the trace metals (Cu, Zn, Fe, Mn) is highlighted on metallograms of the urinary calculi determined by atomic absorption spectroscopy.

## Conclusions

By virtue of its accuracy and expeditiousness, this method proves to be a valuable technique for the analysis of the composition of urinary stones, applicable in the clinical urology (clinical guidelines in the therapeutics and methaphylaxia of urolithiasis).

The identification of the components within urinary stones provides useful information in order to understand the cause of their formation and the prevention of their recurrence. These data are of interest for the clinical guideline in the prophylaxis, therapy and metaphylaxis of urolithiasis.

The various hydration degrees cannot be determined through chemical methods, because these methods cannot distinguish between similar crystalline entities, e.g. COM vs. COD, AP vs. BRS, etc.

FTIR is also effective in the identification of both the crystalline and amorphous nature of a stone, even when analyzing a small amount of sample. A combination of at least two complementary techniques (in our case, chemical methods and FTIR) would be necessary to obtain enough information on the morphology as well as on the molecular and crystalline composition of stones.

The second derivative is used to discern if the stones contain similar compounds in different hydration states.

The FTIR analysis represents a first step for a quantitative analysis of uroconcrements; it may use an equation with the help of the calibration curve or artificial neuronal networks.

FTIR results permit further investigation for the determination of metal concentration by atomic absorption spectroscopy. The metallo-

grams of uroconcrements determined by AAS (consecutive to FTIR analysis) offer a complete view on the calculi biogenesis and information for the management of stone forming patients.

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